# LISTED STOCK GAMETE PRESERVATION

#### SHORT DESCRIPTION:

The Listed Stock Gamete Preservation project seeks to apply cryogenic technology to preserve male gametes from chinook salmon conservation units that are at low levels of abundance and high risk of extirpation. This approach would target chinook salmon populations with ongoing conventional hatchery or captive broodstock artificial propagation programs to preserve and utilize cryopreserved material to enhance genetic diversity. Secondly, this project would establish a gene bank as an insurance policy in case that extirpation of chinook salmon populations does occur. Cryopreservation for hatchery steelhead trout programs that use native brood sources could also be included if desired.

## SPONSOR/CONTRACTOR: NPT

#### **SUB-CONTRACTORS:**

Nez Perce Tribe Department of Fisheries Resources management University of Idaho; Washington State University Paul A. Kucera, Director of Biological Services

Lapwai, ID 83540 208/843-7320 x2

# **GOALS**

#### **GENERAL:**

Maintains biological diversity, Maintains genetic integrity, When utilized with hatchery propagation efforts it could enhance genetic diversity of the propagated population and when gene banked allows for future management and research options.

#### **ANADROMOUS FISH:**

Production

#### NPPC PROGRAM MEASURE:

7.4E

#### **RELATION TO MEASURE:**

The Listed Stock Gamete Preservation project seeks to preserve genetic material from snake River chinook salmon populations (conservation units) that are at low levels of abundance and high risk of extirpation. Preserved germplasm would be available for conventional hatchery and captive broodstock programs and to gene bank material in a salmon germplasm repository for future use.

## **OTHER PLANNING DOCUMENTS:**

The Listed Stock Gamete Preservation project preserves genetic material for future use through gene banking programs identified in the proposed draft recovery plan (NMFS 1995). It also directly applies to the NPPC (1994) and NMFS (1995) directives to initiate captive broodstocks to conserve and move toward recovery of listed salmon populations. Use of cryopreserved material has been recognized as one method of maintaining and promoting genetic diversity in Grande Ronde River chinook captive broodstock populations (ODFW 1996).

TARGET STOCK	LIFE STAGE	MGMT CODE (see below)
SEast Fork South Fork Salmon chinook	Adult	L;S;d
Upper Wallowa River chinook	Adult	L
Minam River chinook	Adult	L;N;d
Wenaha River chinook	Adult	L;N
Upper Grande Ronde R. chinook	Adult	L;S
Imnaha River chinook	Adult	L;S
Big Sheep and Lick Cr. chinook	Adult	L;S
Snake River mainstem trib. chinook	Adult	L;
Lower Salmon River mainstem trib. chinook	Adult	L
Little Salmon River trib. chinook	Adult	L

Rapid River chinook	Adult	L;N
Upper mainstem S. Fk. Salmon River hatchery	Adult	L;S
Mainstem S. Fk. Salmon R. summer chinook	Adult	L;N
Lostine River chinook	Adult	L; S;W.
Johnson Creek chinook	Adult	L;N;
Pahsimeroi River chinook	Adult	L;S
Mainstem Salmon River below Redfish Lk. Cr.	Adult	L;S
Valley Creek chinook	Adult	L
Yankee Fork and trib. chinook	Adult	L;S;d
Herd Creek/upper E. Fk. chinook	Adult	L
Secesh River chinook (includes Lake Creek)	Adult	L;N
North Fork Salmon River	Adult	L;N
Mainstem Salmon River above Redfish Lake Cr.	Adult	L;S
Lemhi River chinook	Adult	L;S
Big Creek chinook	Adult	L;N;d
Camas Creek chinook	Adult	L;N
Loon Creek chinook	Adult	L;N
Sulphur Creek chinook	Adult	L;N
Bear Valley/Elk Creek chinook	Adult	L;N;d
Marsh Creek and Trib. chinook	Adult	L;N
E. Fk. Salmon, mouth to Herd Cr. chinook	Adult	L

# **BACKGROUND**

# Stream name:

All streams and chinook salmon populations listed in NMFS Proposed Recovery Plan for snake River salmon (1995).

# **Subbasin:**

Middle Fork Salmon River; upper Salmon River; Lemhi River; Pahsimeroi River; South Fork Salmon River; mainstem Salmon River tributaries; Grande Ronde River; Imha River; and snake River tributaries.

#### Land ownership:

Both.

# Hydro project mitigated:

The NPPC (1994) directs the Council to develop a program to protect, mitigate and enhance fish and wildlife on the Columbia River and its tributaries ...... affected by the development, operation and management of [hydroelectric projects].......

#### **HISTORY:**

The Listed Stock Gamete Preservation project has previously received a relatively high project ranking through the Columbia Basin Fish and Wildlife Authority. This project was funded in FY 1997 for the first time. The Lower snake River Compensation Plan hatchery evaluations program funded through the U.S. Fish and Wildlife Service has provided a valuable but limited amount of support for this effort since 1992.

#### **BIOLOGICAL RESULTS ACHIEVED:**

None to date. Project to be initiated in 1997.

#### PROJECT REPORTS AND PAPERS:

None to date. Project to be initiated in 1997.

#### ADAPTIVE MANAGEMENT IMPLICATIONS:

This project would provide cryopreserved male gametes for ongoing chinook salmon artificial propagation production programs to enhance genetic diversity in those listed chinook salmon populations. It would allow for preservation of genetic material from salmon populations at low levels of abundance and high risk of extirpation. A germplasm repository would be developed for this purpose as an insurance policy against potential extirpation of the species and to provide for future management and research options. The chinook salmon captive broodstock plan (ODFW 1996) for Grande Ronde salmon populations recognizes the importance of and contains guidelines for the use of cryopreserved semen to maintain genetic diversity of the propagated populations.

# PURPOSE AND METHODS

#### SPECIFIC MEASUREABLE OBJECTIVES:

This project proposes to utilize cryogenic technology to gene bank male salmon gametes from listed snake River chinook salmon conservation units at low levels of abundance and high risk of extirpation. A germplasn repository would eventually be established that would allow safe preservation and storage of cryopreserved germplasm, that would be available to use in conventional hatchery and captive broodstock programs. Material would also be preserved and available for future use. A specific measurable objective would be that cryopreserved material used in artificial propagation programs would result in quantifiable fertilization rates and measurable genetic profiles.

#### **CRITICAL UNCERTAINTIES:**

See Underlying Assumptions or Critical Constraints.

#### **BIOLOGICAL NEED:**

Snake River spring/summer chinook salmon populations have experienced significant decline in population numbers over the past five decades and are now listed as a threatened species. Genetic conservation through population protection and monitoring has not been successful. A genetic resource management approach using cryogenic techniques is recommended for preservation of genetic material from salmon conservation units at high risk of extirpation. These efforts will be needed to preserve and maintain genetic diversity for ongoing artificial propagation programs and to preserve genetic material for future management options. The NMFS draft recovery plan states that "captive broodstock and supplementation programs should be initiated and/or continued for populations identified as being at imminent risk of extinction, facing severe inbreeding depression, or facing demographic risks". The plan further states that "the conservation of local populations or stocks of Pacific salmon and the preservation of their genetic resources is an important goal".

#### HYPOTHESIS TO BE TESTED:

Not research oriented.

# **ALTERNATIVE APPROACHES:**

 $N \setminus A$ 

#### JUSTIFICATION FOR PLANNING:

N/A

#### **METHODS:**

Existing weirs that trap listed adult Snake River spring/summer chinook salmon for artificial propagation will be utilized as sources of male salmon for cryopreservation purposes. Hatchery managers will be coordinated with to determine acceptable periods for sampling to occur. Streams that have had captive broodstock juvenile chinook salmon collected from them will be sampled for collection of wild/natutal adults as will other streams where gene banking will occur. Current limitations include observed fertilization rates of from 0 to 80%. The highest average fertilization rates have been approximately 65% (Mendel - Wash. Dept. of Fish and Wild. - pers. comm.). Mendel also suggested that same day cryopreservation of fresh semen seems to provide much higher fertilization rates when using cryopreserved semen. Cryogenic preservation of female gametes is also a desired product. However, the technology to successfully cryoporeserve female gametes or embryos is currently not available to

fisheries science.

Standardized fish sampling, fish handling and fish anesthesia protocols, consistent with Section 10 permits, will be used to collect and handle adult male chinook salmon for semen cryopreservation purposes. Handling and sampling at hatchery traps would utilize IDFG or ODFW protocols on normally scheduled spawning days. We would also rewquest that male salmon be held as long as possible after spawning so that additional samples may be collected. Abdomens of anesthetized males will be wiped dry and semen samples will be taken into two separate whirl paks. After semen samples are taken adult recovery and semen sample storage procedures occur simultaneously. Adult salmon are immediately tagged, lengths and scales taken, data recorded and the fish placed in a holding pond or pool area where it is assisted until fully recovered. Fin punches are taken and preserved in labelled containers for later DNA extraction. Semen samples are placed in two individually labelled whirl paks, oxygenated, placed in a covered insulated cooler on wet ice on top of newspaper and transported to the University of Idaho (UI), Moscow, ID, and Washington State University (WSU), Pullman, WA. Cryopreservation occurs onsite or independently at the two Universities within a 24 hour period after the samples are taken. Samples are stored in four liquid nitrogen canisters at the UI and WSU.

# PLANNED ACTIVITIES

## **SCHEDULE:**

Planning Phase Start 3/97 End 6/97 Subcontractor NA.

<u>Task</u> 1. Task 2. Task 3. Coordinate gamete preservation activities with state, federal and Tribal management agencies.

Determine chinook salmon populations for germplasm sampling, including sample sizes and number of years sampling will occur. Coordinate with NMFS for necessary Section 10 scientific permits through NMFS. STATEMENT OF WORK CONTAINS OBJECTIVES AND TASKS.

<u>Implementation Phase</u> <u>Start</u> 7/97 <u>End</u> Minimum of 5 <u>Subcontractor</u> University of Idaho; years (TBD). Washington State University.

<u>Task</u> 1. Sampling of adult male salmon would occur at existing chinook weirs used for artificial propagation purposes.

Cryopreservation samples would be collected from approximately July to mid-September at these weir locations. Stream sampling would occur mainly in August and September. Database and sample inventory development would take place from September to December. Report preparation would occur from November through December of each year. Project implementation would continue until a sufficient number of unrelated samples were collected from each population (minimum anticipated project term of five years). STATEMENT OF WORK CONTAINS PROJECT OBJECTIVES AND TASKS.

O&M Phase Start 1997 End Annual. Subcontractor University of Idaho; Washington State University.

<u>Task</u> 1. Storage and maintenance of cryopreserved genetic material will be ongoing after implementation in the summer of 1997.

The germplasm repository for the short-term has been identified as the University of Idaho and Washington State University.

#### CONSTRAINTS OR FACTORS THAT MAY CAUSE SCHEDULE OR BUDGET CHANGES:

Annual abundance in each chinook salmon population. Timing of sampling to collect only spawned-out males. See Underlying Assumptions or Critical Constraints section.

# OUTCOMES, MONITORING AND EVALUATION

#### SUMMARY OF EXPECTED OUTCOMES

#### Expected performance of target population or quality change in land area affected:

Preservation and use of male chinook gametes for use in artificial propagation programs to maintain and enhance genetic diversity. Establishment of gene banks for preservation of genetic material from salmon conservation units at high risk of extinction.

#### Present utilization and convservation potential of target population or area:

Almost all of the listed Snake River chinook nonulations are below threshold numbers of snawning adults in each nonulation/strea

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m and are below 10% of their historical production potential. Some populations such as the upper Big Creek population has experienced three consecutive years of cohort collapse (2 redds in 1994, 1 redd in 1995, and 1 redd in 1996) and is imminent danger of extirpation. This project has the potential for genetic conservation of the male portion of the Big Creek, and other, salmon populations through application of cryogenic technology and preservation for future use. Currently, none of the cryopreserved material has been utilized in artificial propagation programs.

#### Assumed historic status of utilization and conservation potential:

NA

#### Long term expected utilization and conservation potential for target population or habitat:

The desired long term conservation would be to maintain the genetic diversity of the propagated salmon population and wild populations. We would prefer to manage for healthy, self sustaining populations of snake River chinook salmon that would not require the use of cryopreserved genetic material.

#### **Contribution toward long-term goal:**

Genetic material can be used in concert with artificially propagated salmon populations to maintain genetic diversity and the populations ability to change with a changing environment. Cryopreserved material would also be available for future management and research use.

#### Indirect biological or environmental changes:

NA

#### **Physical products:**

Cryopreserved chinook salmon semen wil be stored in 0.5 ml and 4 ml straws in tanks containing liquid nitrogen.

#### Environmental attributes affected by the project:

N/A

#### Changes assumed or expected for affected environmental attributes:

N/A

#### Measure of attribute changes:

N/A

#### **Information products:**

Annual reports will be developed and presented for regional publication and dissemination.

# **Coordination outcomes:**

This project will be coordinated with the state, federal and Tribal salmon managers. coordination should ensure collection of male salmon gametes from existing listed stock hatchery programs.

#### MONITORING APPROACH

Goals will be developed and refined through multi-agency coordination to determine which populations should be addresed and how mny samples should be cryopreserved over the next five year period. Project performance, in terms of number of samples preserved annually, can be easily monitored. Genetic profiles of conventional hatchery reared chinook populations and populations from captive broodstock programs that utilize cryopreserved semen can also be followd to determine if natural and hatchery populations are imilar or dissimilar.

#### Provisions to monitor population status or habitat quality:

Monitoring population status is not a goal of his project. Tribal, state and federal agencies conduct annual chinook salmon redd count surveys and escapement monitoring studies to document the relative abundance of individual salmon populations. Genetic

analysis has been conducted by NMFS to document population profiles and follow them over time. This project will keep an updated computer inventory of all available chinook salmon semen.

## Critical uncertainties affecting project's outcomes:

Additional research needs to be conducted by experts on cryopreservation mehodology (freezing and thawing process) and the fertilization process on a much broader scale. A basin-wide cryopreservation plan should be developed by the salmon mgers.

#### **EVALUATION**

#### **Incorporating new information regarding uncertainties:**

Utilization of new information is an ongoing process that allows project refinement and adaptive management to take place.

#### Increasing public awareness of F&W activities:

This project seeks to preserve genetic material from threatened spring/summer chinook salmon populations from the snake River and to establish a germplasm repository or the same. It is not an information and education program, perse, and is only a stop-gap measure until improvement in minstem survivl conditions alow for self-sustaining populations of salmon.

# RELATIONSHIPS

#### RELATED BPA PROJECT

9604400 Grande Ronde Basin Spring Chinook Captive Broodstock Program Capitol Construction Component 9604300

14-48-301-97502

#### RELATED NON-BPA PROJECT

Lower Snake River Compensation Plan Nez Perce Tribe Evaluation Studies - 1997

#### RELATIONSHIP

Preserved genetic material may be used in spawning protocols to promote genetic diversity.

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Lower Snake River Compensation Plan McCall Hatchery, Lookingglass Hatchery and Sawtooth Hatchery

# **RELATIONSHIP**

This project currently provides limited funds and personnel to collect and cryopreserve male gametes of listed Snake River chinook salmon from selected streams.

# OPPORTUNITIES FOR COOPERATION:

This project would depend on cooperation of those agencies that currently operate adult chinook salmon trapping facilities; mainly IDFG and ODFW. Adult chinook salmon semen was collected in a cooperative effort with IDFG at the South Fork Salmon River fish weir and at Lookingglass Hatchery (Imha River adults) with ODFW in 1996.

# **COSTS AND FTE**

**1997 Planned:** \$110,500

# **FUTURE FUNDING NEEDS:**

## PAST OBLIGATIONS (incl. 1997 if done):

<u><b>FY</b></u>	\$ NEED	% PLAN	% IMPLEMENT	<u>% O AND M</u>
1998	\$140,000	0%	90%	10%
1999	\$145,000	0%	90%	10%
2000	\$150,000	0%	90%	10%
2001	\$155,000	0%	90%	10%
2002	\$155,000	0%	90%	10%

#### OTHER NON-FINANCIAL SUPPORTERS:

U.S. Fish and Wildlife Service, Lower snake River Compensation Plan program; Washington Department of Fish and Wildlife; O

regon Department of Fish and Wildlife.

## **LONGER TERM COSTS:**

It is anticipated that cryopreservation of listed hatchery populations may continue past 2002 if preserved samples are utilized on an annual basis. In adition, if sufficient samples cannot be collected from slmon populations because of low abundance, additional years collections would occur. Operation and mintence of the cryopreserved germplasm would need to occur at some level into the future. Costs are uncertain at this time.

**1997 OVERHEAD PERCENT:** 29.5%

## HOW DOES PERCENTAGE APPLY TO DIRECT COSTS:

Indirect rates apply to a portion of the direct costs, excluding equipment and services.

**SUBCONTRACTOR FTE:** 0.08 FTE - University of Idaho; 0.08 FTE - Wash. State University.